Serum Thymidine Kinase 1 Activity in the Prognosis and Monitoring of Chemotherapy in Lung Cancer Patients: A Brief Report

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Introduction: Thymidine kinase 1 (TK1) is a metabolic enzyme involved in DNA synthesis. Most standard treatment protocols for lung cancer (LC) include cytotoxic agents, which are potential modulators of TK1. We aimed to assess the prognostic significance of serum TK1 activity and its role in monitoring chemotherapy in LC patients.

Methods: TK1 activity was measured using the DiviTum (Biovica) assay in sera from 233 patients with non–small-cell lung cancer (NSCLC), 91 with small-cell lung cancer (SCLC), and 90 with benign lung disease.

Results: TK1 activity was significantly associated with age, performance status, and stage in NSCLC and with stage and weight loss in SCLC. In multivariate analysis, pretreatment TK1 activity, adjusted for performance status, stage, and weight loss, independently affected survival in NSCLC (relative risk =1.45, $p = 0.031$) and SCLC (relative risk = 2.49, $p = 0.001$). In NSCLC patients, adjusted elevated TK1 activity (>100 Du/L) at pretreatment was a significant predictor of treatment failure (odds ratio = 2.55, $p = 0.01$). A small (less than twofold) increase in TK1 activity after the first and second cycle of chemotherapy was significantly associated with treatment failure and poor overall survival.

Conclusions: Elevated pretreatment serum TK1 activity was an independent, adverse prognostic factor, based on survival, in the two main histological types of LC. A small (less than twofold) increase in TK1 activity after the first and second cycle of chemotherapy was associated with treatment failure and poor overall survival.

Key Words: Thymidine kinase 1, NSCLC, SCLC, Prognosis, Monitoring chemotherapy

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One of the most important biological mechanisms of cancer aggressiveness is related to uncontrolled tumor proliferation; its markers have been shown to have prognostic value when measured in lung cancer (LC) patients.[1,2] To take these measurements, however, a biopsy or resection may be required. One such marker is thymidine kinase 1 (TK1), which is the key cytosolic enzyme in the salvage pathway for deoxythymidine monophosphate (dTMP) synthesis; because TK1 is involved in DNA synthesis, it is considered a marker for cell proliferation. Notably, dividing cells release TK1 during mitotic exit, which is mediated by the ubiquitin system.[3] Thus, TK1 activity can be detected in the serum.

Recently, a new highly sensitive assay for the measurement of TK activity in serum has been developed that is based on immobilization of the phosphorylated reaction product by incorporation into DNA.[4] Korkmaz et al.[5] evaluated this assay in a small group of 48 patients with advanced non–small-cell lung cancer (NSCLC) and found a significant correlation between serum TK1 activity and primary tumor maximum standardized uptake, based on 18-fluorodeoxyglucose positron emission tomography scans, and overall survival. The present study was aimed to assess the prognostic significance of the new TK1 assay in the two main histological types of LC, NSCLC and small-cell lung cancer (SCLC).

Although TK1 levels detected in the serum primarily reflect proliferative activity, this enzyme is an important element of a complex system of kinases in the salvage pathway, complementing the main de novo pathway of dTMP synthesis. The activities of the two pathways are coordinated with the transport systems of nucleosides.[6] There are many factors modifying these systems. Most standard treatment protocols for LC include cytotoxic agents that are potential modulators of TK1 activity.[6–8] Therefore, we investigated the usefulness of this assay to monitor treatment with these agents.

METHODS AND PATIENTS

This prospective study was performed on consecutive patients between November 1, 2008, and October 30, 2013. It was approved by the Institutional Ethical Review Board (0441-08-HMO) and included 90 patients with benign lung disease

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Disclosure: S.G. is the inventor of the TK1 assay by DiviTum principle and the founder of Biovica International AB, where his family members hold shares. The other authors state that there are no conflicts of interest regarding the publication of this article.

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TABLE 1. Distribution of TK1 Activity in Patients With Benign Lung Disease or Lung Cancer

		Serum TK1 activity (Du/L)				
Study group	***n***	**Mean**	**Median**	**IQR**	**95th Percentile**	***p* value[a]**
All LC vs. BLD						p <0.001
BLD	90	188	74	29–186	855	
All LC	324	819	155	59–479	3481	
All non—small-cell lung carcinoma	233	468	129	56–344	1,484	
Age (yr)						p = 0.001
≤62	112	509	178	76–514	1,593	
>62	121	424	101	36–241	1,307	
Gender						p = 0.259
Male	151	578	148	60–397	1,705	
Female	82	266	111	42–303	1,135	
Smoking habits						p = 0.561
Nonsmoker	70	929	131	54–410	2, 184	
Smoker/ex-smoker	163	332	129	58–289	1, 496	
Histological type						p = 0.571
Adenocarcinoma	145	536	108	46–341	1,450	
Squamous	59	393	181	60–358	1,520	
Other	29	283	135	42–336	1,509	
Performance status						p = 0.024
0–1	190	302	114	56–292	1,348	
≥2	43	1216	222	82–697	3,138	
Weight loss (kg)						p = 0.469
≤5	94	518	115	58–325	1, 407	
>5	139	400	150	55–373	2, 083	
TNM stage						$p <$ 0.001
I–II	39	153	82	31–152	1, 158	
III	66	234	116	57–224	1, 213	
IV	128	653	181	63–514	1, 711	
All small-cell lung carcinoma	91	1,717	325	85–1478	6,132	
Age						p = 0.278
≤62	44	1, 986	423	102–1555	5, 947	
>62	47	1, 465	195	64–1477	9, 795	
Gender						p = 0.118
Male	66	2,148	398	95–2,273	10,086	
Female	25	577	115	76–697	4, 036	
Performance status						p = 0.177
0–1	54	1, 276	181	60–1,127	6, 104	
≥2	37	2, 298	414	98–1,883	14,586	
Weight loss (kg)						p = 0.013
≤5	56	1, 018	159	50–843	5, 629	
>5	35	2, 828	467	126–3,090	19,195	
Stage						p = 0.007
Limited	32	362	139	82–400	2, 160	
Extensive	59	2, 452	535	95–3,090	12,124	

IQR, interquartile range; TK1, thymidine kinase 1; BLD, benign lung disease; LC, lung cancer.
[a]*p* values derived from Kruskal-Wallis and Mann-Whitney tests.

(BLD), 233 patients with NSCLC, and 91 patients with SCLC. The diagnoses of all lung tumors were confirmed pathologically.

After informed consent was given, serum samples were obtained from all patients, before the start of treatment, and stored at –80°C until analysis. Serum TK1 activity was measured with a colorimetric enzyme-linked immunosorbent assay kit (DiviTum; Biovica International AB, Uppsala, Sweden), as described previously.[9] TK1 levels were expressed in DiviTum units/L (Du/L).

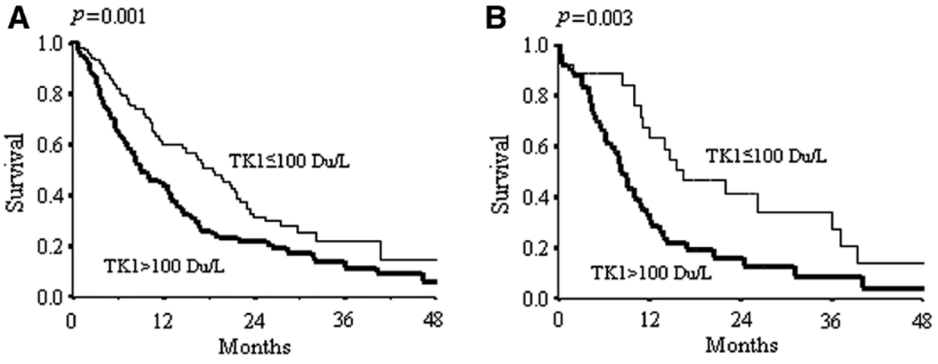

FIGURE. 1. Survival for 233 patients with non–small-cell lung cancer (*A*) and 91 patients with small-cell lung cancer (*B*). Kaplan-Meier estimates according to pretreatment serum TK1 activity. TK1, thymidine kinase.

Two tumor response categories were applied: progressive disease (PD) and nonprogression (NP), including complete remission, partial response, and stable disease.

Statistical Methods

Kruskal-Wallis, Mann-Whitney, Wilcoxon, Spearman's correlation, Fisher's exact test, logistic regression, Kaplan-Meier, and Cox survival models, and receiver operating characteristic curves were used for statistical analyses.

RESULTS

TK1 Activity Assay in NSCLC

Patients with both NSCLC and SCLC showed significantly higher TK1 activities compared with those with BLD (medians 129 and 325 versus 74 Du/L; $p = 0.003$ and $p < 0.001$, respectively). Furthermore, patients with SCLC had a higher TK1 activity than those with NSCLC ($p = 0.017$). A cutoff value between low- and high-serum TK1 was set at 100 Du/L—the closest point on the receiver operating characteristic curve to the optimal sensitivity and specificity in the LC (NSCLC + SCLC) versus BLD model.

In NSCLC (Table 1), the serum TK1 activity was associated with age, performance status (PS), and disease stage. Median survival for patients with normal versus elevated TK1 activity was 18.6 versus 9.0 months, respectively ($p = 0.001$, Fig. 1*A*). Multivariate analysis (Table 2) selected PS, disease stage, weight loss (WL), and serum TK1 as the most important pretreatment characteristics with independent impacts on survival.

Of 233 patients with NSCLC, 199 were treated with chemotherapy. The pretreatment activity of TK1 in PD patients ($n = 74$) was significantly higher than that in NP patients ($n = 125$) (median 183 versus 105 Du/L, respectively, $p = 0.005$). In the logistic regression, the elevated TK1 activity (>100 Du/L), adjusted for stage, PS, and WL, was a significant predictor of treatment failure (odds ratio [OR] = 2.55, 95% confidence interval, 1.25–5.22).

TK1 Activity Assay in SCLC

In SCLC, TK1 levels were associated (Table 1) with disease stage and WL. The median survival for patients with normal TK1 activity was longer than that for those with elevated levels (16.5 versus 8.3 months, respectively, $p = 0.003$, Fig. 1*B*).

TABLE 2. Multivariate Cox's Regression: Relative Risk in Patients with NSCLC ($n = 233$) or SCLC ($n = 91$)

Characteristics	Relative risk	95% CI	*p* value
NSCLC			
Performance status (0–1 vs. ≥2)	3.53	2.35–5.29	< 0.001
Weight loss (no vs. yes)	1.63	1.16–2.28	0.005
Stage (M0 vs. M1)	2.14	1.53–2.98	< 0.001
TK1 (≤100 Du/L vs. > 100 Du/L)	1.45	1.04–2.02	0.031
SCLC			
Performance status (0–1 vs. ≥2)	1.83	1.11–3.02	0.018
Weight loss (no vs. yes)	1.77	1.07–2.92	0.026
Stage (limited vs. > extensive)	1.39	0.80–2.43	0.241
TK1 (≤100 Du/L vs. > 100 Du/L)	2.49	1.41–4.41	0.001

CI, confidence interval; TK1, thymidine kinase 1; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

In multivariate analysis, PS, WL, and serum TK1 independently affected survival (Table 2).

Eighty-five of 91 patients were treated with chemotherapy. The basal TK1 activity in PD patients ($n = 21$, median 730 Du/L) was higher than that in NP patients ($n = 64$, median 197 Du/L, $p = 0.041$). The elevated TK1 activity did not reach statistical significance for the prediction of treatment failure (OR = 1.95, $p = 0.09$).

TK1 Activity Assay in Monitoring Chemotherapy

Patients receiving platinum-based therapy, combined with pemetrexed ($n = 43$), gemcitabine ($n = 13$), vinorelbine ($n = 22$), and etoposide ($n = 18$), were monitored before the first (basal), second, and third cycles of chemotherapy. A significant increase in TK1 activity was observed after the first and second cycles for all applied regimens (Table 3). However, the increase was larger for pemetrexed and gemcitabine (medians, second/first ratio: 6.3 and 8.1, respectively; medians, third/first ratio: 12.4 and 22.8, respectively) compared with vinorelbine and etoposide (medians, second/first ratio: 1.5 and 2.2, respectively; medians, third/first ratio: 1.8 and 1.9, respectively). Patients were divided into two groups: (1) treated with pemetrexed or gemcitabine and (2) treated with vinorelbine or etoposide. TK1 activity significantly increased in PD and

TABLE 3. Changes in TK1 Activity After the First and Second Course of Chemotherapy, According to Regimen

Platinum-based regimen with	Pemetrexed	Gemcitabine	Vinorelbine	Etoposide
Histology	NSCLC	NSCLC	NSCLC	SCLC
Number	43	13	22	18
2nd/1st ratio (median, IQR)	**6.3,** 3.6–20.6	**8.1,** 4.7–30.3	**1.5,** 1.1–2.4	**2.2,** 1.2–11.0
[a]*p* value (2nd vs. 1st)	<0.0001	0.003	0.028	0.008
3rd/1st ratio (median, IQR)	**12.4**, 5.0–38.6	**22.8,** 6.2–53.3	**1.8,** 1.2–3.4	**1.9,** 1.1–14.0
p value (3rd vs. 1st)	<0.0001	0.008	0.026	0.028

TK1, thymidine kinase 1; 1st, 2nd, 3rd, TK1 activity measured before first (basal), second, and third cycles of chemotherapy, respectively; IQR, interquartile range; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.
[a]*p* values were derived from the Wilcoxon signed-rank test.

TABLE 4. Changes in TK1 Activity After the First and Second Cycles of Chemotherapy According to Response

	Platinum/pemetrexed or gemcitabine		Platinum/vinorelbine or etoposide	
Response	PD	NP	PD	NP
Number	11	45	16	24
2nd/1st ratio (median, IQR)	**2.1**, 1.3–15.4	**6.1**, 1.4–15.4	**2.1**, 1.5–4.7	**1.7**, 1.17–10.3
p value (2nd vs. 1st)[a]	0.003	<0.0001	0.030	0.004
3rd/1st ratio (median, IQR)	**1.9**, 1.2–20.4	**6.9**, 2.8–36.5	**1.5**, 1.2–1.7	**3.0**, 1.3–14.3
p value (3rd vs. 1st)	0.028	<0.0001	0.043	0.010

TK1, thymidine kinase 1; 1st, 2nd, 3rd, TK1 activity measured before first (basal), second, and third cycles of chemotherapy, respectively; PD, progressive disease; NP, nonprogressive; IQR, interquartile range.
[a]*p* values were derived from the Wilcoxon signed-rank test.

NP patients in both groups after the first and second cycles of chemotherapy (Table 4). However, this increase was smaller in PD patients than in NP patients, with the exception of one subset of patients in the NP group after the first cycle of platinum with vinorelbine or etoposide. After the second cycle, the ratio in this NP group reached 3.

The study population was split in two groups according to the increase in TK1 activity, using a cutoff of 2.0 (overall median increase in PD patients for all regimens). The increase was more than twofold after the first cycle of chemotherapy in 12 of 27 PD patients (44%) and in 49 of 69 NP patients (71%) ($p = 0.021$). After the second cycle, a more than twofold increase occurred in eight of 23 PD patients (38%) and 58 of 73 NP patients (80%) ($p = 0.003$). Patients with more than a twofold increase in TK1 activity after the first (Fig. 2*A*) and second (Fig. 2*B*) cycles of chemotherapy showed significantly longer median survival compared with those with a less than or equal to twofold increase (17.5 versus 9.9 months, $p = 0.023$ and 19.6 versus 8.3 months, $p = 0.002$, respectively).

We analyzed relationship between TK1 activity and WBC account during chemotherapy. Spearman's correlation coefficients at regimens containing pemetrexed, gemcitabine, vinorelbine, and etoposide were 0.13 ($p = 0.202$), 0.26 ($p = 0.09$), 0.43 ($p = 0.003$), and 0.45 ($p = 0.007$), respectively.

DISCUSSION

The pretreatment serum TK1 activity detected in this study reflects the overall condition (proliferative background) of each individual. The TK1 activity of the lung tumor and its contribution to measured TK1 activity in serum remain unknown. The higher TK1 activity of LC patients versus that of BLD is conceivably due to the higher proliferation of LC. The significant associations of TK1 activity with tumor burden both in NSCLC and in SCLC may indicate, at least in part, its tumor origin.

The DiviTum assay explored in this study demonstrated the clinical importance of measuring TK1 activity as a prognostic marker in NSCLC and SCLC. In both subtypes of LC, elevated TK1 predicted poor survival after the adjustment for disease stage, WL, and PS. The present study is consistent with a previous, retrospective study on TK1 activity, measured by radioenzyme immunoassay.[10] The authors of that study pointed to the promising prognostic value of pretherapeutic TK for overall survival in multivariate analyses of NSCLC patients. Moreover, we showed that elevated pretreatment TK1 activity in NSCLC, after adjustment for stage, PS, and WL, remains a significant predictor of treatment failure with an OR of 2.55.

We find that platinum-based combinations with pemetrexed, gemcitabine, vinorelbine, and etoposide induced a significant increase in TK1 activity. However, the strongest increase was observed in regimens that included pemetrexed or gemcitabine, whereas a less pronounced effect was observed in those that included etoposide or vinorelbine. Topolcan et al.[11] previously observed an increase in TK1 activity in colorectal cancer during chemotherapy with regimens that included 5-fluorouracil (5-FU).

Cytotoxic agents, such as pemetrexed, gemcitabine (through its metabolite, difluorodeoxyuridine monophosphate), and 5-FU, are strong inhibitors of thymidylate synthase and the de novo pathway of dTMP synthesis.[6,12,13] To overcome this effect, cells up-regulate the compensating salvage pathway for dTMP synthesis. The increased activity of the salvage pathway leads to an increased uptake of thymidine

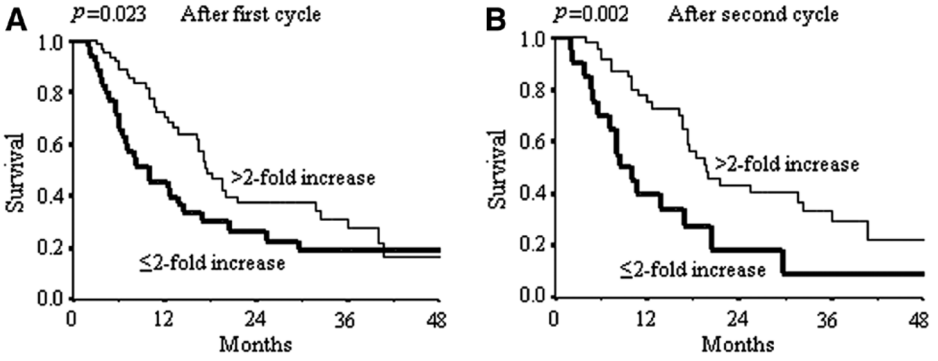

FIGURE. 2. Kaplan-Meier estimates according to the ratio between two measurements of TK1 activity before the first and second (*A*) or third (*B*) cycles of chemotherapy in 96 patients with lung cancer. TK1, thymidine kinase.

through activation of the membrane equilibrative nucleoside transporter 1 (ENT1) delivery system.[6,14,15] Recent experimental data obtained with HeLa cells that highly express ENT1 demonstrated that etoposide was a strong inducer of ENT1 activity and TK1 expression.[8] Activation of the salvage TK pathway and/or the ENT1 system appears to be the main factor explaining increased TK1 activity after treatment with pemetrexed-, and gemcitabine-, or etoposide-containing regimens. At these conditions, TK1 behaves as a metabolic marker, reflecting changes in two pathways of dTMP synthesis.

The increase of serum TK1 activity during chemotherapy may result from metabolic changes both in tumor and normal tissues. This is confirmed by the observation of increased TK1 activity at the use of 5-FU-containing regimens in adjuvant setting in colon cancer patients.[11] The overall increase of TK1 activity might include for example the replication effect from leukocyte. Higher increase denotes higher recovery of the leukocytes and might contribute a better treatment response to treatment. This mechanism might be important at the use of vinorelbine and etoposide. Actually, at these regimens, we observed a better correlation between the TK1 activity and WBC counts during chemotherapy ($r = 0.43–0.45$).

We found that the extent of increase in TK1 activity at the beginning of LC treatment may predict its effectiveness and prognosis. An analysis of changes during chemotherapy showed that a more than twofold increase in enzyme activity was more often found in NP patients than in PD patients after the first and second cycles of chemotherapy. Survival analysis also showed longer survival for patients who had more than a twofold increase in TK1 activity after either the first or the second cycle of chemotherapy. Thus, monitoring by TK1 early during chemotherapy could provide valuable information for detection of nonresponders, thereby preventing unnecessary toxicity.

Our results show that the difference between two response groups was evident already after the first cycle of treatment with regimens containing thymidylate synthase–blocking agents: pemetrexed and gemcitabine. Other mechanisms appear to be responsible for the TK1 activity change at the use of etoposide/vinorelbine, and this may be a reason for delaying increase, which has become evident only after the second cycle.

Further studies are needed to reveal and describe additional factors contributing to overall TK1 condition during chemotherapy and improving associative models.

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